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Some studies on helminth parasites of buff backed heron (Ardeola ibis ibis) with special reference to its role in transmision of Clinostomum complanatum in Beni-Suef Governorate.

S. M. Aboel Hadid[1], H. S. Lotfy[2]

[1] *Parasitology Department, Faculty of Veterinary Medicine, Beni-Suef University and* [2] *Animal health Institute, Beni-Suef , Egypt.*

A number of 50 Ardeola ibis ibis birds were found harboring six nematodes species; Tetrameres species, Microtteramere species, *Synhimantus invaginatus*, Synhimantus equispeculatus, Ascaridia species, Paracamallanus species,and five species of trematodes; *Euclinostomum heterostomum, Nephrostomum ramosum, Apharyngostrigea ibis, Apatemon gracilis* and *Centrocestus armatus*. The most common infection by nematodes was (46%) in which highest infection rate Synhimantus invaginatus recorded (30 %) while the trematode infection was (24 %) and Apatemon gracilis was the most prevalent (16 %). Experimental infection of buff backed heron by encysted metacercaria (EMC) and exysted metacercaria (ExMC) of Clinostomum complanatum from freshwater fish *Tilapia nilotica*, resulted in adult worms formed after 6 days. Where the infection by EMC recorded higher worm burden (14-18 worm) and hatching percent (78%) while the infection by ExMC gave lower worm burden (7-10 worm / bird) and hatching (48 %). In the present study, it is worthy to mention that buff backed heron act as final host model for *Clinostomum complanatum* and this will be helpful in further biological and immunological studies for this trematode to decrease its economic losses in fish intermediate host.

Wild birds are widely distributed due to their ability for adaptation to great climate variations, in an agricultural country like Egypt, man is usually in intimate contact with water, hence there is a potential danger of infestation with parasites of birds (Mahdy and El- Ghaysh, 1998). Heron play an important role in biological control of agricultural enemies such as, insects, mollusks and earthworms. The latter act as intermediate hosts for some trematode parasites after experimental feeding of herons by encysted metacercaria collected from fish (Mahdy, 1991; Tantawy, 1997; Abbas, 1997). Also, many parasites from these birds act as a source of infection for domestic birds by parasites (El-Seify and Abd El-Fattah, 1996). The Buff backed heron (Ardeola ibis ibis) may play a role in transmitting of digenia from freshwater fish as Tilapia nilotica in Egypt, (Abo Essa, 2000) Clinostomum complanatum is a digenetic trematode which naturally parasitizes the throat and oesophagus of pisivorous birds (Yamaguti, 1958). Many species of freshwater fish recorded as the second intermediate hosts of C. complanatum (Aohagi, *et al.,* 1992). Human infection with *Clinostomum complanatum* raw cyst was recorded (Chung, *et al.,* 1995). Clinostomum prevalence rate was 50.2 % in *Tilapia nilotica* in Beni-Suef (Abd El Galil, *et al.,* 2006).

The aim of this study is to investigate the helminth parasites of buff backed heron in Beni-Suef Governorate and its role in transmitting Clinostomum complanatum which may helpful in breake of the cycle and controlling of this trematode.

Material and methods

Survey study. A total of 50 buff backed heron (Ardeola ibis ibis) were collected from different locations in Beni-Suef Governorate, Egypt, from May 2007 to September 2007. The birds were captured and were transferred alive to the labs of Parasitology Dept., Fac. Vet. Med. Beni-Suef Univ. and animal health institute, Beni-Suef. The birds were killed by pithing of the brain and spinal cord or by intracardial injection of saturated magnesium sulphate solution then dissected and the alimentary tract was separated

from mouth opening to the cloaca and the horny layer of gizzard has been splitted and the underneath layer as well as proventriculus were examined for any embedded parasites. Internal organs also collected and were examined for any helminthes. The collected helminthes were washed and separated into trematode, cestode and nematodes. Trematodes and cestodes were pressed, fixed, stained and mounted according to (Wessener, 1968). While nematodes were fixed and mounted according to (Belding, 1965). All helminthes were identified according to (Yamaguti, 1958; McDonald, 1981; Yamaguti, 1961).

Experimental infection.

Collection of Buff backed heron. 15 birds were hunted from the field and reared in metal cages in the lab. Each bird was separated in a cage and the feces were collected and examined daily for about one week at least to ensure that it was free from parasites. The birds were fed water and food ad libitum. The birds (free from parasites) were divided into 3 groups Group A: 5 herons as control negative with out infection, Group B: 5 herons infected with 20 encysted metacercaria of Clinostomum complanatum and Group C: 5 herons infected with 20 exysted metacercaria

Collection of C. complanatum metacercariae. Freshwater fish, Tilapia nilotica, were collected from markets and transferred to the labs. Examination of fish was carried out by dissecting the gills and taken the large size EMC and put in normal saline tell infection of the birds. Some of metacercariae were pressed, stained and mounted

Infection of birds. Group B were infected orally by 20 EMC. Group C were infected by 20 exysted metacercaria (excystation was carried out in 0.1 % acidified pepsin according to Kalantan *et al.,* 1991) While group A were lifted as control negative without infection.

Follow up of the birds after infection. After 6 days, fecal examination was carried out for recording the eggs of adult worms tell the 10^{th} day pi, all birds were killed and the upper part of oesophagus and buccal cavity were removed and separate the worms from it.

Determination of worm burden and fecundity. This was recorded for each group.

The number of worms for each bird in each group was recorded. These worms were put in normal saline and incubated at 37°C for recovering the eggs in 24h, 48h and 72h. Specimens of adult worms were pressed, stained and mounted and identified according to Yamaguti (1958).

Hatching of the eggs. The eggs of each group were collected in a Petri dish (20x3mm) containing normal saline and were incubated at 29°c for 14 days with daily shaking and examination microscopically from day 8 till the appearance of free swimming miracidium.

Results

Survey study. 23 (46 %) birds of 50 buff backed heron were found harboring different helminthes, 46% (23 birds) nematode infection and 24 % (12 birds) trematode infection. 6 types of nematodes were identified, *Synhimantus invaginatus*, *Synhimantus equispeculatus* (Fig. 1) Ascaridia species, Microtetrameres species and Paracamallanus species (Fig. 2). Tetrameres species (Fig. 3) 5 digenetic trematode were collected, *Euclinostomum heterostomum*, *Nephrostomum ramosum* (Fig. 5), *Centrocestus armatus, Apharyngostrigea ibis* and *Apatemon gracili* (Fig.6).

From table (2) the highest infection rate among nematodes was *S. invaginatus* (30 %) while the lowest one was Paracamallanus species (2 %). In Table (3), *Apatemon gracilis* was the most prevalent trematode (16 %) while the lowest one was *Euclinostomum heterostomum* (2 %). The mixed infection by more than one type of infection was recorded (24%).

Experimental infection. Adult worms of *Clinostomum complanatum* were recovered from the birds after 10 days where the eggs appeared in feces from 6^{th} day post infection. Worm burden, worm fecundity and hatchability of eggs were studied for each group (Table 4 & Fig. 6).

From table (4), it was evident that, the infection of heron by encysted metacercaria of *Clinostomum complanatum* gave average number of worms per bird (12-18worms). The number of eggs per worm was 4750-5250 eggs and these eggs when incubated gave hatchability rate 78 %, where the hatching began from day 8 of incubation at 29°C and completed after 2 weeks.. While the infection by exysted metacercaria gave lower worm burden (7-10 worms / bird) where eggs per worm were higher (16500-21900 egg / worm) and hatching rate of these eggs was lower (48 %).

Table (1): Incidence of helminth infection of the examined birds.

Helminth	No. Examined birds	No. infected birds	Infection rate (%)
Nematodes	50	23	46
Trematodes	50	12	24
Mixed infection	50	12	24

Table (2): Incidence of nematodes infection among 50 examined birds.

Nematode	No. Inf. birds	Inf. percent (%)
Microtetrameres species	5	10
Tetrameres species	2	4
Synhimantus invaginatus	15	30
Synhimantus equispeculatus	10	20
Ascaridia species	5	10
Paracamallanus species	1	2

Table (3): Incidence of trematodes infection among 50 examined birds

Trematode	No. Inf. birds	Inf. percent (%)
Euclinostomum heterostomum	1	2
Nephrostomum ramosum	5	10
Centrocestus armatus	4	8
Apatemon gracilis	8	16
Apharyngostrigea ibis	6	12

Table (4): Worm burden, fecundity and hatching of each group.

Group	Worm burden/ bird	Worm fecundity	Hatchability %
B	12-18	4750-5250	78
C	7-10	16500-21900	48

Fig. (1): A- S. equispeculatus i- Ant. end ii- Post. end male iii- Post. Female
B- S. invaginatus i- Ant. end ii- Post. end male iii- Post. Female (X 100)

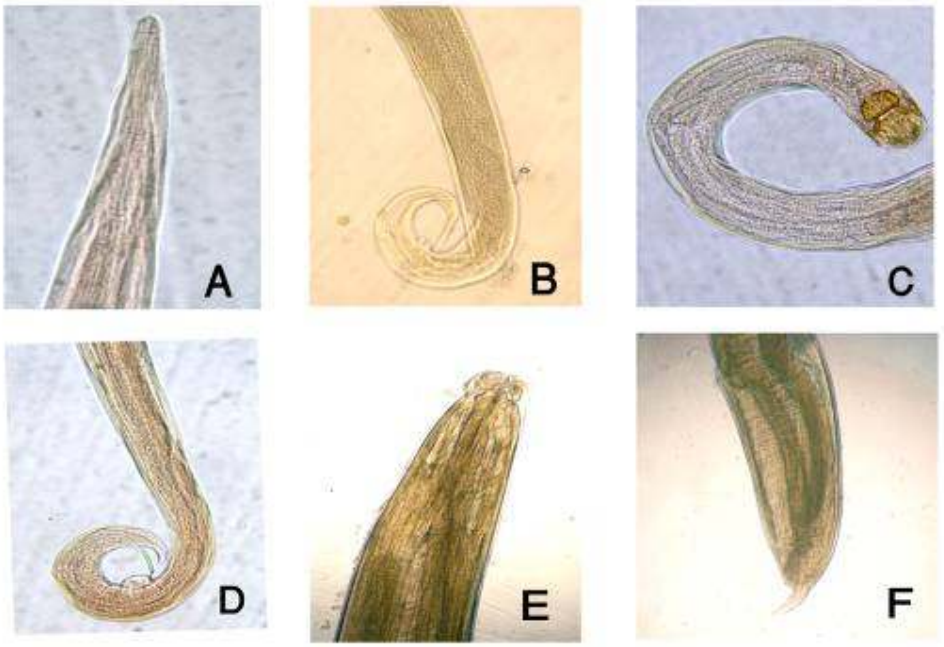

Fig. (2): A&B- Microtetramere species i- Ant. end ii- Post. end male (X200)
C&D- Paracamalanus species (Ant. & Post. ends) (X 200)
E&F- Ascaridia species (Ant. and Post. ends) (X 40)

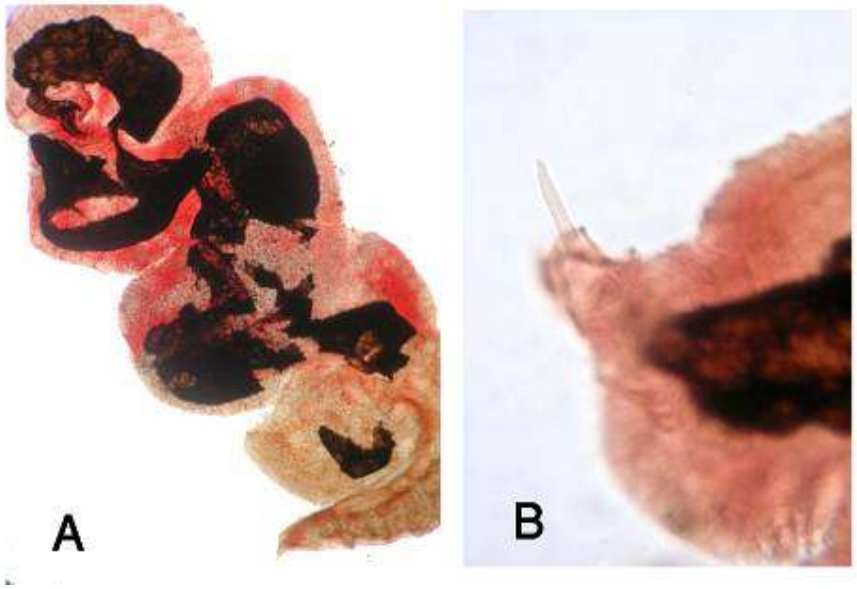

Fig. (3): Teterameres species A- Adult worm B- Ant. End (X 40)

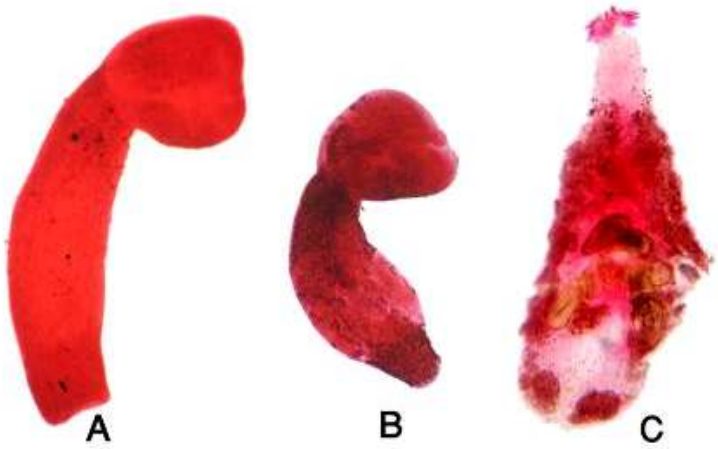

Fig. (4): A- Apharyngostrigea ibis (adult worm) (X40), B- Apatemon gracilis (adult worm) (X40), C- Centrocestus armatus. (adult worm) (X100)

Fig. (5): A- Euclinostomum heterostomum (adult worm), B- Nephrostomum ramosum (adult worm)

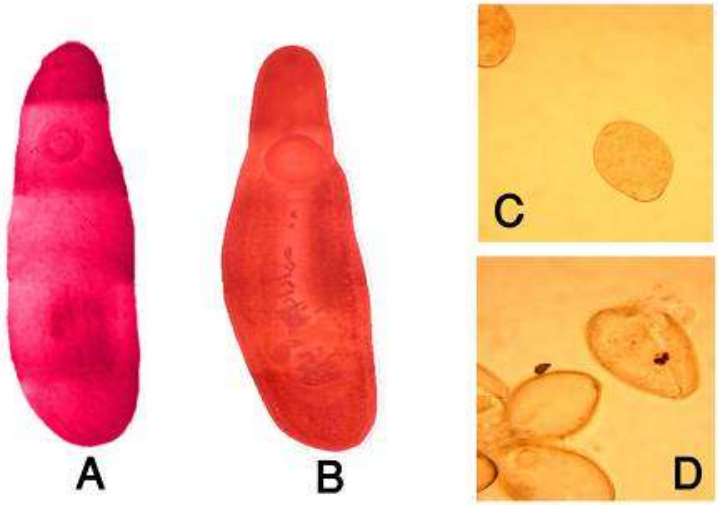

Fig. (6): Clinostomum complanatum: A- Exysted metacercaria, B- Adult worm, C- Immature eggs, D- Mature eggs (containing miracidium).

Discussion

Wild birds constitute a potential hazard for domestic species and human being due to spreading of infections specially the parasitic ones (Khalifa and El Naffar, 1983; Abd El-Fattah,1996).These birds feed on arthropods, mollusks, fishes, reptiles and rodents in which many of them act as intermediate host for helminthes (Joseph, 1979; Ahmed, 1994).

The present study revealed that the infection rate of helminth parasites was 46 % among the examined buff backed heron (Ardeola ibis ibis). These results were nearly agreed with Hassan and Abd El Aal (1999) 41.8 % in Suez canal area and Sinai Peninsula, Egypt, while, it was lowered than Ahmed (1994) 100% in Zagazig area; Mahdy and El Ghaysh (1998) 85 % in Giza area. In the other hand, our results were higher than that recorded by Hegazi (1978) 21.7 % in Tanta area; Abd El Fattah (1996) 38.6 % in Kafr El-Sheikh area. These variations may be due to the different localities.

Among different types of helminthes, nematode infection recorded the most common parasites among the examined birds (46 %). This disagreed with Hegazi (1978) 2.6 %; Ahmed (1994) 25.9 %. But, go in parallel with Mahdy and El Ghaysh (1998) 85%; Hassan and Abd El Aal (1999) 70 %. These nematodes were *Synhimantus invaginatus, Synhimantus equispeculatus*, Ascaridia species, Paracamallanus species and Microtetramere species and Tetrameres species. The most prevalent nematode species were *S.invaginatus* (30 %) These results are similar to that obtained by Mahdy and El Ghaysh (1998); Mousa and Mahdy (1998); Hassan and Abd El Aal (1999). Mean while it was found that the lower prevalent one was Paracamallanus species, and this agreed with Mahdy and El Ghaysh (1998).

The prevalence of trematode infection was 24 % which was lowered than that reported by Ahmed (1994) 37.6 %; Amer and Desoky (1995) 33.3 %. While, it was higher than that of Abd El Fattah (1996) 3.7 %; Hegazi (1978) 3.8 %; Hassan and Abd El Aal (1999) 14.6 %.

The digenetic trematodes were *Euclinostomum heterostomum*, *Nephrostomum ramosum, Apatemon gracilis, Apharyngostrigea ibis* and *Centrocestus armatus*. This finding go in parallel with that recorded by (El-Naffar and Khalifa 1975; Hassan and Abd El Aal,1999; Abo Essa, 2000; Lotfy, 2003).

The results of the experimental study showed that buff backed-heron took the infection by *Clinostomum complanatum* encysted and excysted metacercariae and the adult *C. complanatum* formed in the upper part of oesophagus within 6 to 8 days post infection. On the contrary, Hassan and Abd El Aal (1999), fed *C. complanatum* encysted metacercaria to buff backed heron but they were not found any adult worm. On the other hand, Amer, *et al.,* (1988) collected *C. complanatum* from mouth cavity of chickens after 4-6 day post infection. In addition, Mahdy (1991) found immature flukes after 2 days from infection of chicks. In the author opinion and in accordance to Kalantan, *et al.,* (1991), age, sex and breed of chicken affect on susceptibility to *C. complanatum*, both percent susceptibility and recovery of the worms decreased with increase in the age of the host. Mature male chickens were found more susceptibility than females. The single comb white Leghorn (SCWL) was found to be susceptible than others. The biological studies (worm burden and worm fecundity) was studied in this work to participate in the knowledge about of *C. complanatum* biology cycle.

It is worthy to mention that buff backed heron was proved to be a final host for C. complanatum. In author opinion this will be helpful in determining the parts of *C. complanatum* life cycle and participate in biological control of it or studying the immunology of this parasite where buff backed heron distributed all the country and can be used in these studies.

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بعض الدراسات على ديدان ابوقردان الطفيلية مع التركيز على دوره فى نقل طفيل الكلينوستومم كومبلينيتم فى محافظة بنى سويف

بفحص ٥٠ طائر من طيور ابوقردان وجدت ستة أنواع من الديدان الاسطوانية و هى ميكروتترامير اسبيشس وتترامير ز اسبيشس و سيناهيمنتس انفاجينيتس و سيناهيمنتس اكوسبكيولاتس و اسكاريديا ابوقردان و الباراكاملينس اسبيشس. و كانت سيناهيمنتس انفاجينيتس الاكثر شيوعا (٣٠ %) .وجدت ايضا خمسة ديدان ورقية هى ايوكلينوستومم هتيرواستومم و نفرواستومم راموسم, افرينجواستيريجا ايبس و اباتيمون جراسيلس و سنتروسيستس ارميتس و كانت الاصابة بالاباتيمون جراسيلس هى الاعلى (١٢ %). اما بالنسبة للعدوى التجريبية لابوقردان باليرقات المتحوصلة و اليرقات خارج الحويصلة لطفيل الكلينوستومم كومبلينيتم و المجمعة من اسماك البلطى النيلى الطازجة ، فان العدوى حدثت بالطريقتين و اعطت ديدان بالغة بعد ٨ ايام و بمقارنة العدوى بالطريقتين وجدت ان طريقة العدوى باليرقات المتحوصلةا تعطى عدد اكثر من الديدان ونسبة فقس البيض اعلى من الاخرى. لذا فان الدراسة أفادت فى معرفة جزء من دورة حياة طفيل الكلينوستومم كومبلينيتم و بالتالى المساهمة مستقبلا فى دراسته بيولوجيا و مناعيا للحد من انتشاره فى الاسماك لما له من اثر اقتصادى.